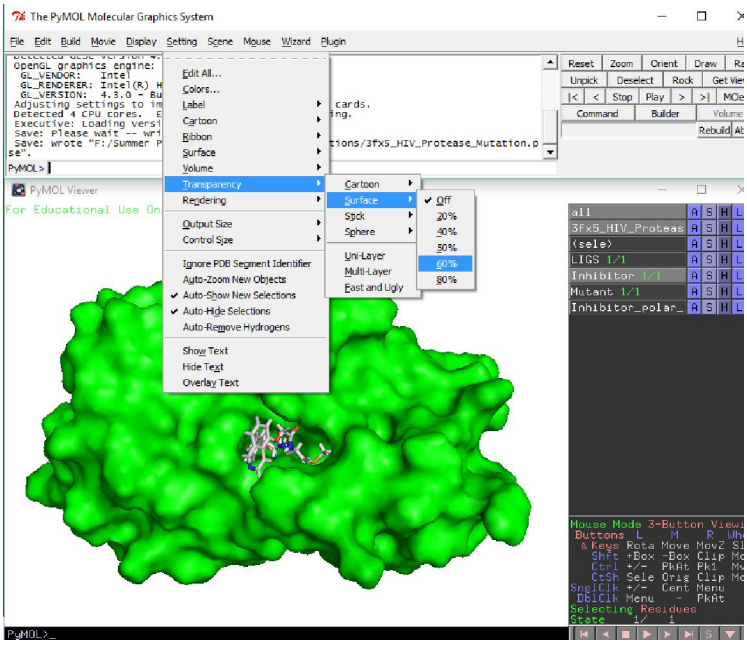
WA5- Active Sites and Mutations

Download and then open the file 3fx5_HIV_Protease in PyMOL. This is a HIV protease protein, bound to an inhibitor. HIV protease is one of the key proteins within the HIV virus. As a virus, HIV will reproduce inside the body, and this requires it to produce certain necessary proteins. The HIV protease enzyme is one of the essential proteins which helps HIV to replicate in the body, by assisting with protein replication. The purpose of an inhibitor is to stop this process from happening. If an inhibitor is successful in doing this, then it has the potential to be used as a drug to treat HIV/AIDS.

1. Protease will cut larger polypeptides into shorter, specialised ones. Using the command **HIV_Protease>S>as>surface**, show the surface of the protein structure. Using your knowledge of inhibitors, explain how the inhibitor shown stops the enzyme from working effectively.



2. Set the transparency of the surface using the command **Setting>transparency>surface>60%** on the top toolbar.
   Now select the **Inhibitor_polar_conts_** to show the interactions between the inhibitor and the enzyme. How is the inhibitor held in the active site of the enzyme?

Produced by Adam Stubbs at Newcastle University as part of a summer outreach project.

3. What would happen to the effectiveness of the inhibitor, and of the HIV protease enzyme, if there were more of these interactions in place?

4. Why is it important that the interacting groups, the ones which bind to the enzyme, are in the specific locations that they are? What would happen if they were replaced, or changed position slightly?

5. The inhibitor contains multiple chiral centres. Why is it important to consider these when designing a drug?

6. Using the command **Setting > Transparency > Surface > off**, remove the transparency of the enzyme. We will now consider the effect of mutations in a protein.
   Enzymes are proteins made from chains of amino acids. These amino acids are coded for by DNA. What could happen to the structure of the enzyme if the DNA code is changed slightly?

7. In PyMOL, a mutated enzyme has been created. This has been done by duplicating the enzyme, but replacing just one of the amino acids in the sequence with a different one. In this case, a valine has been replaced with a tryptophan**. Select Mutant in the right-hand pane to overlay the mutated enzyme onto the healthy HIV protease and the inhibitor**. How does the mutated enzyme compare to the healthy one, and what impact could this have on the inhibitor?

8. Mutations happen randomly in the DNA, and this mutation could have occurred anywhere in the amino acid sequence – the primary structure. The mutation is equally likely to occur anywhere else in the molecule. What effect would this have on the overall function of the enzyme, and the effectiveness of the inhibitor, if the mutation happened away from the active site?

9. Why is it useful to use computational models such as PyMOL when designing drugs and inhibitors such as this?

10. The inhibitor is shown to be a very potent inhibitor. What else should be considered before producing and using this drug on the mass market as a treatment to HIV?